REMARKS

Reconsideration of the rejection of claims 1-4 under 35 USC §112, second paragraph, is respectfully requested in view of the amendments to the claims.

Reconsideration of the rejection of the claims under 35 USC §§102(b), 102(e) is respectfully requested in view of the amendments to the claims and the following remarks.

As a preliminary matter, it is submitted that none of the references, either alone or in combination with the others, teaches or suggests bioremediation of contaminated water from the containment area of a nuclear power plant by colonized microorganisms found in such water. These microorganisms cannot be cultured, whereas the prior art cited by the examiner relies on methods using organisms that can be so cultured. Thus, nothing in the cited art teaches or suggests the claimed invention.

USP 6,719,902 (Alvarez) concerns compositions, methods, and apparatus for the removal of nitrogenous and halocarbon pollutants from environmental sources including agricultural areas, soil, ground and surface water, sewage, sludge, landfill leachates, and wastewater. In particular embodiments, this patent discloses and claims compositions comprising zero-valent iron and hydrogenotrophic bacteria for use in removing target contaminants by a synergistic combination of abiotic and biological reductive mechanisms. This is very different from the claimed invention wherein oligotrophic radioactive waters of nuclear pools are decontaminated. The inventive procedure does not require adding microorganisms. Moreover, this method concentrates and eliminates mainly 60-cobalt, a compound not claimed by Alvarez el at.

US-6,080,572 (Hard) relates to gram-negative sulphate-reducing bacteria that under anaerobic conditions reduce sulphate to sulphide by using methanol as a source of carbon and energy. The disclosure refers to methods of decontaminating sulphuric-acid and metal-containing and radioactively contaminated water. In the claimed invention, oligotrophic radioactive water is characterized by the absence of sulphates, which, as a result, cannot use sulphate-reducing bacterial strains. Moreover, the process typically takes place under aerobic conditions, and no source of carbon needs to be added to the bioreactor for the procedure to be carried out.

In US-5,393,426 (Raskin et al.) terrestrial plant roots are used to absorb, concentrate, and precipitate metal from the aqueous solution (phytoremediation). The metals that can be accumulated and precipitated by the plants come from a variety of heavy metals or radioactive metals. The column used is preferably made of an inert material such as polyvinylchloride,

polytetrafluoroethylene (i.e. Teflon), or glass. The metal-containing solution may be aerated. In the claimed invention, oligotrophic radioactive water of nuclear pools can be decontaminated by using the microorganisms that already exist in this radioactive water. The column used is preferably of an inert material such as stainless steel or titanium. The invention can be used inside the fuel building where it is not permitted to cultivate or grow plants. As well, the claimed invention is preferably a closed process and does not cause environmental problems.

US-4,043,936 (Francis et al.) uses biological denitrification of nitrate solutions anerobically in an upflow column having as a packing material a support for denitrifying bacteria.

US-4,826,602 (Revis et al.) utilizes *Pseudomonas maltiphilia* ATCC 53510 to reduce the concentration of ionic species of heavy metals in aqueous waste solution in the presence of some nutrient medium that can satisfy the nutritional requirements of *Pseudomonas maltiphilia* ATCC 53510 cell cultures. In the claimed invention radioactive water of nuclear pools is decontaminated by the same microorganisms that are found in the water. Furthermore, it is not necessary to add a nutrient medium to satisfy the nutritional requirements of the microorganisms.

US-4,861,519 (Tusa et al.) relates to a method and apparatus to reduce the quantity of low activity organic wastes from nuclear power plants. This is carried out by anaerobic fermentation in a bioreactor. The gases produced in the decomposition process are conducted from the methane stage to a gas burning stage. This has nothing to do with the claimed invention

US-5,895,832 (Eccles) refers to a process for treating contaminated materials. This specific process for removing metal, especially heavy metals or contaminants from bulk particulate material, such as land or soil, is carried out using biochemical processes. The process comprising the steps of treating a body of the said medium with microbially produced sulphuric acid so as to solubilize the metal species as a metal sulphate; treating the leached metal sulphate by a bioprecipitation process that converts the said sulphate into an insoluble sulphide; separating hydrogen sulphide produced during the bioprecipitation from the insoluble metal sulfide; and oxidizing the separated hydrogen sulfide to form a reusable source of a sulphur-containing ingredient. The claimed invention relates to the decontamination of radioactive water and not to treating land or soil, and it is not possible to apply the Eccles's process to radioactive waters of nuclear pools.

US-5,422,268 (Rusin) discloses a biological process for recovering plutonium from soil using an iron-reducing bacterium, *Bacillus circulans* NRRL B-21037 in a growth medium. In the claimed invention radioactive water of nuclear pools is decontaminated by microorganisms that are found in the water. Moreover, it is not necessary to add a nutrient media to satisfy the nutritional requirements of the microorganisms.

US-5,948,259 (Deguitre et al.) refers to a process and apparatus for treating oils and solvents contaminated by radioactive substances. This has no relevance to the decontamination of radioactive water of nuclear pools inside the fuel building in nuclear power plants.

DE 4433413 A1 Patent is based on the bioremediation of acidic water from the mining industry. As this water contains large quantities of sulfates, metals, and radionuclides, the objective is to prevent it from seeping to surface or underground water levels. The procedure is carried out *ex situ* in a bioreactor where the microorganisms are immobilized on a type of support that is not specified. The microorganisms are sulfate-reducing bacteria or SRB that, when in the presence of a carbon source added to the system, anaerobically reduces the sulfate content and produces sulfur instead. This sulfur reacts with the metals that come into contact with it, thus reducing its concentration. The carbon source used is methanol or any other inexpensive nutrients of the like that are available. In the claimed invention, the microorganisms are immobilized in a biofilm that is formed on inert metallic materials. No carbon source or nutritive medium needs to be added the bioreactor in order for the procedure to be carried out. Moreover, the process can be performed in a continuous way, whereas the German patent is performed in batch.

USP 5,447,629 corresponds to a patent developed by French inventors on an apparatus used to purify a liquid effluent containing metals and/or radionuclides. Noting teaches the claimed invention even though its objective is also to eliminate radionuclides and other contaminants such as metals. The "purifying" apparatus disclosed in the '629 patent is a transparent bioreactor that is exposed to light and that immobilizes living cells of photosynthetic microorganisms. In the claimed invention, the bioreactor is preferably made of a metallic material and none of the immobilized microorganisms are photosynthetic, as the bioremediation process of the liquid effluent can be carried out in complete darkness. The apparatus of the '629 patent uses photosynthetic microorganisms, that could be bacteria, microalgae, or cells isolated from high plants; although for purposes of simplification, only living microalgae are referred to

in the claims of the patent, thus differentiating it from a previous patent that used dead microalgae. These microalgae are powerfully bioabsorbant. In our apparatus, microalgae are not used. The bioremediation process of the '629 patent can be carried out in a continuous and semi-continuous way, and in some occasions require a nitrogen-enriched liquid culture medium, and CO_2 is also added to the same system so that the microalgae can grow. In the claimed invention, the system is carried out in a continuous way and a culture medium is not used in the bioreactor, although it is possible to create a biofilm on the metal by winding it into balls and placing them in a culture medium in the laboratory before the material is placed in the bioreactor.

The article by Ashley and Roach has no influence on any of the claims of the present patent, but it does make it more interesting. Basically, this article *proposes* the use of another already commercialized technique that eliminates heavy metals to eliminate radionuclides from aqueous systems, but makes no mention to any technical information or any other previous experiment. It is based on a work hypothesis. The authors talk of the *possibility* of immobilizing microorganisms on pellet or granular-shaped supports placed in a bioreactor. In our patent, the procedure is completely different and the microorganisms form a biofilm on metallic balls. These authors (quoting work carried out by others) talk about immobilizing certain microorganisms in polyacrilamide-gel beads because of their capacity to bioabsorb uranium. This does not interfere in any way with any of the claims of the patent being requested. In a basic and very general way, as the journal is fundamentally addressed to chemists, the article talks about the basic microbiological principles that have prevailed in the selection process of microorganisms when used in specific applications, as well as the genetic engineering techniques used to improve microorganisms that were initially isolated. This system is currently used in the pharmaceutical, beer-brewing, and chemical industries, etc. to enhance production processes, and would not have led one of ordinary skill in the art to the claimed invention.

US 5,487,834 is based on a bioremediation procedure via the biodegradation of contaminants *in situ*. In order to carry this out, the '834 patent proposes (taking the biodegradation of hydrocarbons in the environment as a previous reference), adding microorganisms or pumping nutrients into the subsurface to stimulate the growth of indigenous bacterial populations. These microorganisms known as metanotrophic. Of these the *Methylosinus trichosporium* bacteria are especially able to produce enzymes intracellularly, known as methane monooxygenases (MMOs), which act as biocatalyzers in the degradation of

fundamentally aromatic organic chemicals. The patent's authors, after having presented laboratory and *in situ* studies on biodegradation of organic contaminants, suggest that they can be applied to other contaminants such as metals or radionuclides without mentioning any previous experiment or information on the matter. In the claimed invention, the bioremediation of water contaminated with radionuclides uses a bioreactor where the microorganisms are immobilized when biofilms are formed. This is completely different from freely adding microorganisms or adding specific nutrients to the medium that will be bioremediated as the existing patent proposes.

Accordingly, it is submitted that this application is in condition for allowance, and an early indication thereof is respectfully requested. Please charge any deficiency and credit any excess to deposit account 50-1088.

Respectfully submitted,
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